

Sphingomyelins and Cell Signaling

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INTRODUCTION

Eukaryotic organisms, in addition to certain prokaryotes and viruses contain sphingolipids. In mammalian tissues, these can be categorized into ceramides, cerebroside, gangliosides, sulfatides, and sphingomyelins. Sphingomyelins are particularly abundant in nerve tissue of higher animals and play an important role in the function of signaling in cells. This paper will discuss how sphingomyelins function in cell signalling and the pathway that accomplishes, the functions that this pathway.

REVIEW OF LITERATURE

Sphingolipids

Ohanian and Ohanian reviewed sphingolipids and their involvement in cell signaling and reported that sphingolipids are involved in many different cellular processes such as differentiation, cellular senescence, apoptosis, and proliferation (1). These lipids are identified by their sphingoid backbone. Sphingolipid biosynthesis starts with the condensation of serine and palmitoyl CoA which then forms 3-ketosphingosine which is further reduced to dihydrosphingosine. Amide linkage adds a fatty acyl group to form dihydroceramide, which is directly converted into ceramide by introducing a trans-double bond linking the fourth and fifth carbon of the sphingoid base. Different headgroups can be added to ceramide in order to form sphingolipids which are more complex. The most simple of these is ceramide-1-phosphate which is formed by ceramide kinase. Through adding phosphorylcholine to ceramide, which was brought from phosphatidylcholine by sphingomyelin synthase, sphingomyelins are formed.

Sphingomyelins

Membrane properties

Sphingomyelins are important components of the outside leaflet of the plasma membrane of cells (2). Sphingomyelin content in mammalian tissue ranges from 2-15% of total organ phospholipid. Sphingosine containing a trans double bond between C₄ and C₅ is the most common base in the sphingomyelins of mammals, but other bases may be found as well,

though they are present in smaller amounts. The composition of the sphingomyelins acyl chain varies between different tissues, however they are commonly found to be unusually long, making them asymmetrical. There is a large occurrence of saturated amide-linked acyl chains which have an average of 0.1-0.35 cis-double bonds per molecule. When present, these cis-double bonds are generally located a large distance from the interface. In the interfacial region, a free hydroxyl on C₃, the trans-double bond linking C₄ and C₅, along with the amide group can be found. The hydroxyl and amide groups can work as hydrogen bond acceptors and/or donors, while the amide carbonyl in sphingomyelins can only perform as an acceptor of hydrogen. This gives sphingomyelins the unique ability to create both intra- and intermolecular bonding of hydrogen. Sphingomyelins have a cylindrical molecular shape, which makes them form bilayers when they are hydrated to minimize free energies. The T_m of most natural sphingomyelins is close to physiological temperature.

Sphingomyelin synthases

Sphingomyelin synthesis is mediated by a phosphatidylcholine:ceramide cholinephosphotransferase, or sphingomyelin synthase (3). Huitema et al worked to classify the enzyme that is responsible for sphingomyelin synthesis in animals. Sequences from a set of 27 candidates of sphingomyelin synthases from humans, mice, and *C. elegans* were put together into three major families of protein, CSS1, CSS2, and CSS3. Open reading frames in humans, mice, and *C. elegans* were cloned to examine whether any of the three protein families contained sphingomyelin synthase. A large quantity of sphingomyelin synthase genes were found to be a universal feature in sphingomyelin generating organisms. It was

found that sphingomyelin synthase (SMS) 1 and 2 are transferases that need two fatty acid chains on the choline-P donor molecule so that they can be efficiently recognized as a substrate, and that they directly and distinctively recognize the choline head group on their substrates. Human SMS 1 and 2, in addition to being SM synthases, are transferases, able to use phosphatidylcholine (PC) and SM as donors of phosphocholine to produce PC or SM, depending on the relative concentrations of diacylglycerol and ceramide as acceptors of phosphocholine. This means that human SMS 1 and 2 contain many enzymatic characteristics. Northern blotting found that a low abundant 3.8 kb transcript for SMS 1 in human kidney, brain, liver, heart, stomach, and muscles was present. In these tissues, a major 1.9 kb transcript for SMS 2 was found. This suggests that human SMS 1 and 2 are encoded by genes expressed in a wide variety of places. Synthesis of sphingomyelins takes place in the Golgi complex and plasma membrane. There has been some debate as to whether this also occurs in the endosomes. Immunofluorescence microscopy was used to test this. SMS 1 was found to be associated with the Golgi apparatus, however, whether SMS 2 passes through endosomes or not was unable to be determined. The actions of SMS 1 and 2 were believed to be SM synthases as a result of this study.

Yan et al performed a study on the overexpression of sphingomyelin synthase (4). Previous studies had revealed that the HDL blood cholesterol levels have a negative correlation with the development of atherosclerosis, and epidemiological studies show that levels of sphingomyelin in the plasma is an independent risk factor for the disease. They evaluated the connection between cellular sphingomyelin levels and metabolism of cholesterol. To do this, they created two line of cells that overexpressed SMS 1 or 2 using the

Tet-off expression system. They discovered that SMS 1 or 2 overexpression in Huh7 cells, considerably brought up the levels of intracellular sphingomyelin ($P < 0.001$), cholesterol ($P < 0.05$), and apolipoprotein A-I ($P < 0.001$). This overexpression also brought down levels of apolipoprotein A-I in cholesterol in the cell culture medium which meant that there was a flaw in both of these processes. Their findings were able to indicate that the manipulation of sphingomyelin synthase activity could manipulate the sphingomyelin metabolism, as well as the metabolism of cholesterol, and apolipoprotein A-I.

The Sphingomyelin Cycle

Activation

Dobrowsky et al investigated the activation of the sphingomyelin cycle (5). Nerve growth factor activates the sphingomyelin cycle, which creates the second messenger ceramide. When investigating the effect of nerve growth factor on the activation of the sphingomyelin cycle in T9 cells, addition of a ceramide analog permeable to cells copied the effects of nerve growth factor on the inhibition of cell growth and process formation. This pathway was found to be mediated by $p75^{NTR}$ (the low affinity NGF receptor) in T9 cells and NIH 3T3 cells that overexpress $p75^{NTR}$. Epidermal growth factor was given the ability to activate the sphingomyelin cycle by the expression of receptor- $p75^{NTR}$. The data found in this study show that $p75^{NTR}$ is able to independently signal the trk neurotrophin receptor ($p140^{trk}$). It also found that ceramide may be a mediator in the biology of neurotrophin.

Function of ceramide

As briefly mentioned earlier, ceramide has an essential role in the metabolism of sphingolipids. Hannun has reviewed the sphingomyelin cycle and the second messenger function of ceramide (6). Ceramide is a precursor for other complex sphingolipids. A phosphocholine headgroup is added to ceramide to form sphingomyelins. Studies done with $1\alpha,25$ -dihydroxyvitamin D_3 and TNF- α show that the sphingomyelin pathway is started by the interaction of TNF- α and the 55-kDa receptor. Cytosolic neutral sphingomyelinase is then activated. The sphingomyelin cycle is completed with the resynthesis of sphingomyelin, presumably by the transfer of the choline phosphate headgroup from phosphatidylcholine to ceramide. The ceramide potentially has multiple functions as second messenger such as regulation of cell growth and differentiation, promoting cytotoxicity, and regulation of protein secretion.

Involvements of the sphingomyelin pathway

Muscle satellite cells

Nagata et al studied the significance of sphingolipids signaling for the entry of muscle satellite cells into the cell cycle (7). Skeletal muscle regenerates because of the existence of satellite cells. It was found that sphingosine-1-phosphate provokes the satellite cells to enter the cell cycle. Sphingosine-1-phosphate synthesis from sphingomyelin was found to be the prominent pathway responsible for the initiation of cell division. Upon the initiation of the stimulus, sphingomyelin in the inner leaflet of the plasma membrane is cut by N-SMase, and

consequent metabolism results in an increased level of sphingosine-1-phosphate. When the sphingosine-1-phosphate generation was inhibited, muscle regeneration was disturbed.

Strackowski et al studied how insulin sensitivity relates to the sphingomyelin pathway in human skeletal muscle (8). Previous in vitro studies have revealed that insulin resistance may be attributed to ceramide in the sphingomyelin signal pathway. This study was done to look at the fatty acid content in ceramide and sphingomyelin in the muscle of humans and assess their association with insulin sensitivity. The study was done on 27 male participants with a normal glucose tolerance. They performed euglycemic-hyperinsulinemic clamps and biopsies of vastus lateralis muscle on the subjects. Thirteen sphingomyelins and ceramides were identified according to fatty acid residues. It was found that insulin sensitivity was connected to the total ceramide content ($r = -0.49$, $P=0.011$), as well as ceramide containing palmitic ($r=-0.48$, $P=0.011$), palmitoleic ($r= -0.45$, $P=0.019$), myristic ($r= -0.42$, $P=0.028$), and nervonic acid ($r= -0.39$, $P= 0.047$). Infusion of intralipid/heparin caused a lowering of insulin sensitivity by 24.73% and a decrease in ceramide content by 47.81%. This study indicated that the sphingomyelin pathway in muscle may be an key aspect that determines the development of insulin resistance in humans.

Stress Induced Apoptosis

Many living organisms are able to eliminate selected cell types through apoptosis as a regulatory mechanism for development, growth, and differentiation. Peña et al offer a review of studies done on sphingomyelin pathway and this mechanism (9). There are different physiologic stimuli and environmental stresses that cause the biochemical pathways of

apoptosis to start. In certain cell systems, the second messenger ceramide acts as the initiator for cell differentiation or proliferation, but it can also signal apoptosis (cell death) in other systems. The activation of neutral sphingomyelinase has been linked to the extracellular regulated kinase cascade and pro-inflammatory responses. Acid sphingomyelinase has been linked to the stress activated protein kinase/c-jun kinase cascade and apoptotic responses. Stress from the environment (for example UV and ionizing radiation) directly acts on membranes to activate acid ph-dependent sphingomyelinase (ASMase), while cytokine receptors signal ASMase activation through motifs called death domains. Ceramide analogs mimic the effect of stress in cells and induce apoptosis.

Using sphingomyelins

Predicting memory impairment

Mielke et al conducted a study on the relationship between sphingomyelins and ceramides on memory impairment (10). Because of the link between sphingomyelins, ceramides, and apoptosis, they can be gauges of neurodegeneration and Alzheimer's disease progression. They examined the starting serum sphingomyelins and ceramides from one hundred 70-79 year old women over a period of 9 years, as predictors of memory impairment. These blood levels were examined relative to cross sectional and incident impairment on HVLT (Hopkins Verbal Learning Test) -immediate and –delayed memory recall. It was found that sphingomyelins and ceramides differed in relation to the timing of HVLT-delayed impairment. Low levels were linked with cross-sectional impairment, and high levels forecasted incident impairment in asymptomatic participants. When using logistic

regression to inspect the cross-sectional relationship between lipids and brain function, each tertile increase in total sphingomyelin (odds ratio 0.32, $p=0.047$) was linked with lowered chances of impairment on HVLT-delayed (23 out of 89 total events). The results of this study propose that ceramide and sphingomyelin in serum vary according to the timing of the start of memory impairment and can be good biomarkers that memory impairment may occur, which is something observed early in the progression of Alzheimer's disease.

CONCLUSIONS

Sphingomyelins are just one of the various types of sphingolipids. It has been found that sphingomyelins are important for cell signaling. Sphingomyelin synthases 1 and 2 have been identified and thought to be the two primary synthases for the synthesis sphingomyelins. Ceramide serves as a second messenger in the sphingomyelin pathway which is activated by nerve growth factor. Sphingomyelins are highly involved in the human muscle cells. Their signaling allows the muscle cells to repeatedly regenerate. The sphingomyelin cycle is also involved in the stress induced apoptosis of cells. Sphingomyelins have also shown to be useful predictors of memory impairment.

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